Remarks:

Rejection Under 35 U.S.C. 103

1. Amended Claims.

The Examiner states in the Final rejection that the Applicant argues limitations that are not present in the claims. In response thereto, Claim 1 has been amended to read "and a fluorescence protein gene of a target cell differentiated from an embryonic stem cell strongly expressed by the said first promoter" thereby overcoming the Examiner's rejection with respect to limitations. The terms 'strongly expressed by the said first promoter' is disclosed in the last paragraph of column 'Disclosure of the invention' in the specification.

Furthermore, the Examiner states that Applicant's previous arguments with respect to "the introduced gene of interest is maintained in the cell nucleus under episomal conditions, this gene is stable and the expression of this gene is not influenced by the host's chromosome" are not persuasive because it is not recited in the claims. Presently amended Claim 1 now reads "and a recombinase-expressing gene are arranged in this order from a 5' side, respectively, with an adenovirus vector as an episomal form into an embryonic stem cell" thus reciting the advantages of using adenovirus, as taught in the specification. The terms 'with an adenovirus as an episomal form' is supported and disclosed in column 'Example 3' of the specification of the present invention.

2. Response to the Specific References:

A. Vallier's reference

The inventors of the present invention found for the first time that a target cell differentiated from an ES cell was not visualized as a result of low activity of a promoter by ligating a promoter of a gene expressed specifically in a tissue such as Nkx2.5 into EGFP gene directly. The present invention was made based on this fact; the EGFP gene is strongly expressed in only a target cell differentiated from an ES cell. The usefulness of this invention is characterized by identifying and purifying a target cell differentiated from an ES cell.

Further, the Examiner states that "The Vallier's reference discloses an *in vivo* method for selectively isolating and visualizing embryonic stem cells after transfection with a vector

encoding the EGFP under the control a constitutively activated promoter." However, in Vallier's reference a target cell differentiated from an ES cell is not identified and purified.

The data in Vallier's reference only shows that the DNA constructs in Figs.1 A and B become DNA construct in Fig. 2 A with 4'-OHT treatment by changes of three marker genes of LacZ(B-geo), EGFP and hAP. Because the EGFP gene on 5' side of DNA construct shown in Fig. 2 A is excised by Cre-recombinase, a target cell differentiated from an ES cell is not identified and purified by using the EGFP gene as a marker.

The Cre-ER^{t2} introduced into the ES cell is described in the right hand column of page 2467 of references 11 (Feil et. al BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATION 237, 752-757(1997) and 12 (Indra et. al, 4324-4327, Nucleic Acids Research, 1999, Vol.27, No.22). Also, the Cre-loxP system in the references submitted with the IDS on April 22nd, 2008, is different from that in the present invention.

B. Rybkin reference

In the Rybkin reference, transgenic mice are generated by the use of ES cells. It is a common general technical knowledge in this field, and is clearly shown in the textbook "Recombinant DNA, 2nd Edition" by James D. Watson, Michael Gilman, Jan Witkowski and Mark Zoller (Published by Science American Books (1992) that the DNA of interest is integrated into the ES cells for generation of transgenic mice. If the DNA of interest is not integrated, then it cannot express in an ES cell for the long term and transgenic mice cannot be generated. Accordingly, the DNA of interest is integrated to target cells. The relevant sections of the text are attached hereto.

Furthermore, in the same textbook, page 256 of Chapter 14 states that "Foreign genes become integrated in the chromosomes of recipient animals." And, at page 257 of Chapter 14, "Foreign DNA can become stably integrated into germ line cells." Also in Chapter 14 at pages 257-259 "Embryonic stem cells can carry foreign genes."

4. Ong's reference

As argued previously, the DNA of Ong is integrated into the target cells. The word

'integrated' is specified in the Ong abstract. Accordingly the DNA of interest is integrated to target cells. In response to the Examiner's rejection, Claim 1 has been amended and now overcomes the Examiner's rejection with respect to Ong.

5. Yamamoto's reference

The Examiner states that Yamamoto complements Vallier, Ong and Rybkin and further claims that there is no reason to believe that an *in vivo* delivery of a target molecule using an adenovirus vector would not work *in vitro.* The Applicant respectfully disagrees. It is common general technical knowledge in this field (and is described in a the above referenced textbook) that the Cre-expressing adenovirus *in vivo* is indeed different from Cre-expressing adenovirus *in vitro.*

Therefore, as set out above, the present invention would not have been motivated by the references cited.

Conclusion:

Applicant believes that this communication is intended to be fully responsive to the outstanding Office Action and fully addresses the Examiner's rejections and now places the application in condition for allowance. No new matter has been added.

Please charge any fee deficiency or credit any overpayment with respect to this paper and/or this application to Apex Juris Deposit Account No. 50-2069. Should Examiner believe further discussion regarding the above claimed language would expedite prosecution they are invited to contact the undersigned at the number listed below.

In view of the above, Applicant respectfully submits that each of claims 1-4; 6, 7, 14-18, 21, 24,27, 30, 33, 34 and 36 recites statutory subject matter that is novel and new, is subject matter of the present invention and is fully supported in the disclosure of the present invention, and therefore respectfully requests that claims 1-4; 6, 7, 14-18,21, 24,27,30,33,34 and 36 be found allowable and that this application be passed to issue. No new matter has been included.

If for any reason, the Examiner determines that the application is not now in condition for allowance, it is respectfully requested that the Examiner contact the Applicant's undersigned attorney at the indicated telephone number to arrange for an interview to expedite the disposition of this application.

In the event this paper has not been timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fees for such an extension, together with any additional fees that may be due with respect to this paper, may be charged to counsel's Deposit Account No. 50-2069, **referencing docket number 042-301.**

Respectfully submitted,

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